

any air bubbles that do form. In some embodiments featuring three heater zones, the vents can span a distance from the outermost 95 C denaturation heater to the middle heater, with an individual vent for each amplification track. However, those skilled in the art that positioning of the vents is flexible and will depend on the particular layout of the amplification zone.

[0171] In some embodiments, all or part of the top plate can be coated with a hydrophilic, oleophobic material to absorb excess aqueous reagents while excluding oil, such that the oil in the chamber stays evenly distributed through the chamber (s). In some embodiments, this hydrophilic and oleophobic material prevents oil from flowing into select regions but allows passage of aqueous fluids and air, for example surrounding the entry ports, such that oil is prevented from venting up through the entry ports.

[0172] While in most cases, the top and bottom plates are electronically insulated from each other, in some optional embodiments, materials, such as one or more conductive sponges, can be used to electrically connect the top plate to the bottom PCB substrate.

[0173] The contacts between the bottom and top plates are generally bonded together with an oil and temperature resistant adhesive as is generally known in the art. That is, the bottom surface of all or part of the top plate is bonded with the edges and dividers of the PCB bottom plate. Similarly an adhesive on the top surface of the top plate is used to bond the top plate with the LRM.

Liquid Reagent Modules

[0174] In addition to the bottom substrate and the top plate, the cartridges of the invention additionally comprise a liquid reagent module (LRM) for the delivery of liquid reagents to the reaction chamber(s) of the cartridge. This fits on top of the top plate for delivery down through the entry ports in the top plate to delivery fluids to the bottom substrate, with the housing covering some or all of these three layers. As is generally described herein, the present systems rely on a combination of dried reagents on the bottom substrate combined with liquid buffers that are dispensed by the activation of deformable storage compartments, generally referred to herein as “blisters”, “blister packs” or “blister vessels”. The activation of the blisters is generally done using actuators that exert pressure (e.g. mechanical pressure, air pressure, etc.) on the blisters to force liquid out of the blister, through the top plate and onto one or more locations (e.g. one or more electrowetting pads) on the bottom substrate as is more fully outlined below. In some embodiments, the actuators for blister activation are contained in the bays of the instrument into which the cartridges fit, e.g. a mechanical actuator or an air pump that puts pressure on the deformable blisters. The instant Figures shows a biochip cartridge with the blister zones exposed, as well as one with a trademark label (which can include a barcode) on the top that hides the blister pressure zones. In general, the blisters can be either sealed at a specific location, generally at the site of the fluid channel leading to the holes in the top plate, such that the seal can be broken. Other embodiments use a uniform blister material that can only rupture in a particular location (e.g. above a hole in the top plate, for example). The blisters can also be ruptured using contained lances that are triggered by the external mechanism. In addition, the blisters can be ruptured and the reagents held in place as needed until dispensing; e.g. the release of the reagents can be time separated from dispensing the reagents.

[0175] Air motivation of fluid can be supplied by an air pump external to the consumable, or alternately supplied by an air blister within the consumable. In either configuration, air or an inert gas is used to push fluid through the system.

[0176] In general, the LRM contains a plurality of blisters that are made of a deformable material that preferentially collapses upon the application of suitable pressure; that is, the materials used to form blisters do not return to their starting shape when the pressure is removed, as this could cause backflow of the applied reagents. In addition, the blisters may be used once (a single application of pressure is done during the assay) or a number of times (e.g. multiple aliquots of reagent are delivered to either a single location or multiple locations during the assay run). For example, one of the blisters contains the immiscible fluid(s), as described herein, which is applied generally as a first step after the sample has been loaded and the cartridge has been inserted into the instrument. In some embodiments, the cartridge can be fabricated with the oil already dispersed on the surface, although this may not be preferable for storage considerations. Alternatively, some blisters are actuated repeatedly for dispensing of the suitable liquid reagent to different pads on the substrate; for example, when the sample droplet is not used to suspend the dried reagents, reconstitution buffer can be added to the different dried reagent pads prior to merging the reagent droplet with the sample droplet. Alternatively multiple blisters containing the same liquid reagent can be used, although this is not generally preferred. This redundancy may be used to deliver the same reagent to multiple locations in the rest of the disposable.

[0177] In addition to the immiscible fluid blister, other blisters are used as generally depicted in the Figures. For example, lysis buffer (which in some cases can be water for hypotonic lysis, or can be a commercially available lysis buffer, such as those containing chaotropic salts such as guanidinium salts, and/or high/low pH, and/or surfactants such as sodium dodecyl sulfate (SDS), Tween 20, Triton-X, etc. is contained within a blister that is activated to add lysis buffer to the sample. In some cases, the lysis buffer optionally comprises reagents to disrupt undesired enzymatic activity, such as DNase and RNase activity, which are then removed during the bead capture/elution process (although these can be separate reagents, either dried or liquid, that can be added as needed depending on the target analytes and the assay). Other suitable blister vessels include, but are not limited to, blister (s) containing binding buffer for binding of nucleic acids or other target analytes to capture beads, blister(s) containing wash buffer, blister(s) containing the elution buffer (again, which can be water in some embodiments) to elute the adsorbed nucleic acids off the beads, blister(s) containing appropriate reconstitution buffer(s), etc. Air blisters (e.g. containing air or other gases) can also be used to exert pressure on either other blisters or down through ports to facilitate free liquid movement (e.g. liquid not subjected to electronic movement such as electrowetting). In some embodiments, blisters can dispense liquid reagents into other blisters, as one method of mixing reagents, or to recover the vast majority of a valuable reagent by flushing it out of a blister.

[0178] In some embodiments, the blisters of the LRM are located directly above the location for dispensing, where the exit port of the blister is aligned with the ports of the top plate such that the fluid is dispensed directly below the exit port of the blister. Alternatively, the LRM may include one or more channels to allow multiple aliquots of reagent liquid to be